The Origin of Hexapoda: a Developmental Genetic Scenario

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ABSTRACT

The similarities and differences in the body plans of Crustacea and Hexapoda are discussed and analyzed. Based on phylogenetics and comparative developmental genetics, an evolutionary scenario is proposed on the origin of Hexapoda. Basically, it is assumed that Hexapoda would derive from an ancestor bearing crustacean-like characters. Changes in the pattern of expression of *Hox* genes would be correlated to the observed changes in body plans. Secondarily, the "master gene" *Distalless*, required in limb formation, would have passed under the control of the *Hox* genes. This change would have produced the reduction of leg pairs to a number of three, and would be fixed by terrestrial life. The interest of the interplay of comparative developmental genetics and of phylogenetics is stressed, the former being able to propose realistic evolutionary scenarios at the genetic level, the second to critically test them.

RÉSUMÉ

L'origine des Hexapodes : un scénario de génétique du développement

Le présent article décrit un scénario concernant l'origine évolutive des Hexapodes. Sur la base de phylogénies récemment proposées, à partir de données tant moléculaires que morphologiques, on suppose que les Hexapodes dériveraient d'un ancêtre de type Crustacé. Dans la lignée conduisant aux Hexapodes, un changement se serait produit dans le profil d'expression des gènes *Hox*. Ce changement rend compte des différences de plan du corps entre Crustacés et Hexapodes. Dans un second temps, le gène *Distalless*, responsable de la morphogénèse des appendices, serait passé sous contrôle des gènes homéotiques. Cela aurait provoqué la réduction à trois du nombre de paires de pattes. Ce changement aurait été fixé par l'habitat terrestre des Hexapodes. La rencontre nécessaire de la génétique du développement et de l'analyse phylogénétique est soulignée, la première permettant de proposer des scénarios d'évolution vraisemblables au niveau génique, la seconde permettant de tester la validité de ces scénarios particuliers.

DEVELOPMENTAL GENETICS AND EVOLUTIONARY BIOLOGY

Until the birth of the "Synthetic theory", genetics (Mendelism) and evolutionary biology (Darwinism) developed separately and sometimes were in conflict. Similarly, for a time, genetics and embryology were quite separate fields. This time is now behind us, as shown by the recent recognition of developmental genetics by the Swedish Academy, awarding the Nobel Prize to the founders of the developmental genetics of *Drosophila*, E. LEWIS, C. NUSSLEIN-VOLHARD and E. WIESCHAUS (see DEUTSCH *et al.*, 1995). In parallel, developmental biologists today direct their research to the molecular and genetic mechanisms of development. However, for epistemological

and also obvious practical reasons, up to now, both developmental biology and genetics have focused on a small number of model organisms.

The time is now ripe for a new field: comparative molecular developmental genetics, at the crossroads of evolutionary biology, genetics and embryology (LAWRENCE & MORATA, 1994). The growth of this new field is indeed currently boosted by i) the discovery of the homeobox twelve years ago and the widespread distribution of homeobox genes (GEHRING, 1994) which opened our minds to the idea of the evolutionary conservation of developmental genes across the Metazoan kingdom, and ii) the extraordinary power of newly developed tools in molecular biology.

Developmental genetic information can be of high phylogenetic value. It yields new available characters, that can be integrated like other characters in phylogenetic analyses. In my opinion, the type of data generated by this new discipline deserves more particular attention, due to their dual quality of being both genetic *and* developmental. As genetic, because the genome is the place where the whole history of life is engraved, as a consequence of the Darwinian principle of "descent with modification", which must be the basis of all phylogenies. As developmental, since embryologic and larval characters may be of higher taxonomic (*i.e.* phylogenetic) value than adult ones, according to Darwin himself, (DARWIN, 1859), as he experienced in his study of the crustacean cirripedes. Without taking for granted GARSTANG's sentence that "*ontogeny creates phylogeny*" (for a comment see DEVILLERS & TINTANT, 1996), it should be stressed that evolutionary radiations in the Metazoa correlate with changes in the body plan, which is the result of genetically controlled developmental processes.

The interface of developmental biology and phylogenetics could be the promise of a "New synthesis" in the theory of evolution (GILBERT, 1991).

THE PURPOSE OF THE PRESENT ARTICLE

Here I emphasize the juxtaposition of developmental biology and phylogenetics by proposing an evolutionary scenario of the origin of Hexapoda based mainly on our current, and I must confess, still scarce, knowledge of the developmental genetics of crustaceans and insects.

So doing, I include myself in the tradition of developmental genetics. M. AKAM (AKAM *et al.*, 1988) synthetized the hypotheses of LEWIS (1978) on *Hox* gene evolution and the developmental model proposed by RAFF & KAUFMAN (1983), itself derived from SNODGRASS, and proposed a scenario in which insects derived from an annelid-like ancestor by progressive steps. These included onychophoran- and myriapod-like states, correlated with an increase in the number of *Hox* (homeotic) genes (see below for more details on *Hox* genes).

Given the improvements of our knowledge in both phylogenetics, which now rejects any close relationship between annelids and arthropods, (EERNISSE *et al.*, 1992; KIM *et al.*, 1996) and of comparative molecular developmental genetics, which has shown that a large gene complex was likely already present at the origin of arthropods (see below and Fig. 1), the main features of AKAM's 1988 scenario are presently unvalidated. Nonetheless, this model has been quite useful in two ways: summarizing the current knowledge of different scientific fields and stimulating new experimental research.
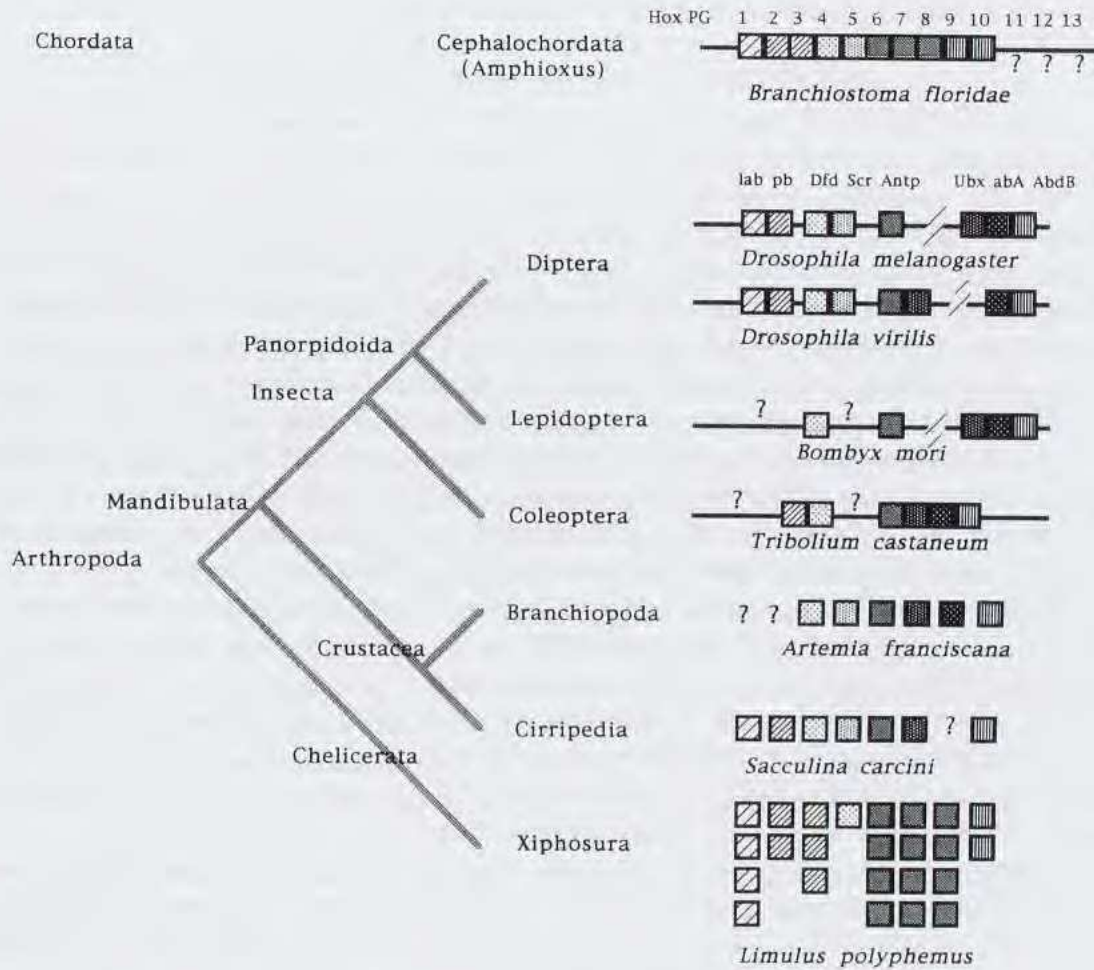
FIG. 1. — The evolution of *Hox* genes. **Right** : The *Hox* genes of a selection of arthropod species are represented, after available data in the literature. Each *Hox* gene is represented by a square of a distinct color. Gene names are given above the squares for *Drosophila melanogaster*. Genes of the same color in different species are assumed to be orthologous. A question mark means that the gene of interest has not been reported to date, which does not prove that it is not present. The squares are lined on a bar when genetic linkage has been demonstrated by genetic and/or molecular evidence. A split in this bar indicates a split of the *Hox* complex at this position. The absence of a bar in a given species indicates that up to now there is no direct experimental evidence for the grouping of the *Hox* genes in a complex in this case. For sake of clarity, non homeotic genes physically present within the gene complexes of *Drosophila* are not represented. **Left** : A likely phylogenetic tree of the arthropod species represented is drawn. **Top** : For comparison, the *Hox* gene complex of the *Amphioxus*, probably close to the ancestral chordate one, is drawn (GARCIA-FERNANDEZ & HOLLAND, 1994). The numerals on top of the diagram indicate the conventional chordate paralogy group of *Hox* genes (SCOTT, 1992).

If the scenario I present here had only a portion of the value of AKAM's in this regard, I would be fully satisfied. But my main purpose is to describe an example of what can be done in the field, rather than to present a completely realized scenario.

THE BODY PLAN OF CRUSTACEANS AND HEXAPODS

The body plans of Crustacea and Hexapoda show a great parallelism. Both include unsegmented parts at each end, the acron and telson, and three groups of segments, or *tagmata*, in between. The first tagma is the *head*. The cephalic parts of crustaceans and hexapods are very similar to each other.

The second tagma of crustaceans is called the *pereion*. It includes a number of segments, extremely variable according to the various sub-classes or orders. Each pereionic segment bears a pair of ventral appendages. This is a strong rule: only very few and derived crustacean species do not bear appendages on *all* pereionic segments. The number of pereionic segments, although quite variable, is never three. The second tagma of Hexapoda is the *thorax*, always composed of three segments. Each hexapod thoracic segment bears a pair of legs, hence the name. The presence of the three pairs of legs is again a strong rule, loss of legs always being a derived state. On the other hand, the structure, function and even the mere presence of wings or wing-like appendages, are quite variable and distinctive traits among Hexapoda orders.

The third tagma of Crustacea is called the *pleon*. Again, it is composed of a highly variable number of segments, ranging from none at all in Cirripedia to more than twenty in some Notostraca (*Triops*). Depending on the crustacean order, or sometimes even genus, the pleon may or may not bear appendages. The third tagma of Hexapoda is the *abdomen*, composed of a fixed number of 11 segments. Only Collembola and some Diptera have a reduced number of abdominal segments. At the adult stage, the last two abdominal segments may be reduced. The hexapod abdomen does not bear locomotory limbs.

In Crustacea, the male genitalia are always located on the segment that marks the border between the pereion and the pleon (the last pereionic segment, sometimes called the genital segment). The female genital aperture may be located in the same position, or more anteriorly, depending on the Order. In Hexapoda the genitalia are always located in a posterior position: in males probably primarily on the 9th or 10th abdominal segment, in females probably primarily behind the 7th sternum.

Despite the differences just described, the gross partitioning of the segmentation pattern of both Crustacea and Hexapoda into three parts leads to the proposal that the three tagmata of these two classes are homologous structures, following the corresponding order along the anterior to posterior axis. The homology between crustacean and hexapod heads leaves little doubt, keeping in mind that the exact number of head segments is still controversial, and that there are some characteristic differences between the cephalic appendages of Crustacea and Hexapoda. The homology between the second and third tagmata is less obvious but up to now has been generally agreed upon, and it is common in most classical zoology textbooks to find the term "thorax" used as a synonym for the crustacean pereion, and "abdomen" for the pleon.

PHYLOGENETIC RELATIONSHIPS

The monophyly of the Hexapoda is rarely contested. Among Hexapoda the relationships of the various orders are in general agreed upon. It is also the case for the basal groups, the

relations of which are becoming better clarified thanks to recent advances in cladistic analyses (KRISTENSEN, 1991, 1995; MINET & BOURGOIN, 1986).

The situation is just the reverse for Crustacea: some sub-classes are clearly monophyletic, such as the Malacostraca. But the relationships between groups are still very uncertain. Thus, it is difficult to decide which of the crustacean sub-classes or super-orders represents the sister group of Malacostraca. These problems are probably related to the diversity of body plans among Crustacea, and to the deep ancestry of the stem taxa of each lineage. Recent molecular phylogenetic studies have not shed light on this question (ABELE *et al.*, 1992).

Another major problem concerns the relationship between Hexapoda and Crustacea. Myriapoda, Hexapoda and Crustacea together belong to a "Mandibulata" clade, distinct from the other arthropod classes, namely the extinct Trilobita and the extant Chelicerata. The Mandibulata are generally assumed to be monophyletic. The most "classical" view is to consider the Myriapoda as the sister-group of Hexapoda. They could be united into a clade called "Tracheata", on the basis of several similarities interpreted as synapomorphies, including the uniramous morphology of the limbs and the presence of tracheae, hence the name.

However, recent molecular phylogenetic studies have led to the hypothesis that Hexapoda would be more closely related to Crustacea than to Myriapoda (BOORE *et al.*, 1995; FRIEDRICH & TAUTZ, 1995; TURBEVILLE *et al.*, 1991).This would lead to the rejection of the clade "Tracheata" comprising Hexapoda and Myriapoda. Although still controversial, this phylogenetic hypothesis is supported by morphological arguments, mainly based on the striking similarity between the patterns of wiring of the nervous system between Crustacea and Hexapoda, which differs from that of Myriapoda (WHITINGTON *et al.*, 1991; OSORIO *et al.*, 1995; OSORIO & BACON, 1994). In addition, certain traits, previously taken as synapomorphies grouping Myriapoda with Hexapoda, such as the presence and morphology of the trachea, are now considered as convergent adaptations to terrestrial life. It has even been proposed that hexapods might be evolved from a crustacean ancestor, thus making the class Crustacea paraphyletic (FRIEDRICH & TAUTZ, 1995; NIELSEN, 1995, p. 162). (For a contradictory view on arthropod phylogeny, see FRYER, 1996).

COMPARATIVE DEVELOPMENTAL GENETICS

The body plan of metazoans is under the control of a particular set of genes, the homeotic or *Hox* genes, highly conserved in structure and function in triploblasts at least. Each *Hox* gene is a "master gene" or "selector gene" which determines, in a specific domain along the anterior posterior axis of the body, a particular morphogenesis programme during development. *Hox* genes were first discovered in a hexapod, the fruit fly and favourite genetic model *Drosophila melanogaster*. They are now known in a variety of insects. Recent studies on the branchiopod *Artemia* (AVEROF & AKAM, 1993) and on two cirripede species, (E. MOUCHEL-VIELH & J. S. DEUTSCH, unpublished) show that the crustacean ancestor possessed a complement of the same eight typical *Hox* genes as found in *Drosophila* and other insects (Fig. 1). In particular, Crustacea possess clear orthologues of both *Drosophila Ubx* and *abdA* genes. This is not the case in the annelid *Holobdella*, where the *Hox* genes *Lox2* and *Lox4* are closer to each other than to either *Ubx* or *abdA*, indicating that the *Ubx/abdA* duplication postdated the divergence between Annelida and Arthropoda (WONG *et al.*, 1995) . Unfortunately, data from the single chelicerate studied until now, *Limulus*, do not permit a determination of the precise relations of

orthology with the other arthropod homeotic *Hox* genes, because of the short length of the fragments cloned and of the tetraploidy of the *Limulus* genome (CARTWRIGHT *et al.*, 1993). Hence, it is not yet possible to assess whether the *Ubx/abdA* duplication is shared by all members of the phylum Arthropoda or by Mandibulata only (Fig. 1).

The variation in body plan between Crustacea and Hexapoda cannot be attributed to a simple change in the number and/or structure of the *Hox* genes. However, striking differences are observed between the pattern of expression of homologous *Hox* genes between Hexapoda and Crustacea. Each *Hox* gene is expressed in a specific domain along the anterior-posterior axis of the animal during development. The specific domain of activity of each *Hox* gene actually results from this spatial specificity of expression and from the combinatorial interactions between the *Hox* genes' products. In *Drosophila* the four more "posterior" *Hox* genes are *Antennapedia (Antp), Ultrabithorax (Ubx), abdominalA (abdA)* and *AbdominalB (AbdB)*. The specific domain of *Antp* is the thorax, the *Ubx* domain comprises the posterior half of the second thoracic segment, the third thoracic and the anterior half of the first abdominal segment, the *abdA* domain expands from the second to the fourth abdominal segments, and *AbdB* reigns over the most terminal abdominal segments. Besides details, this pattern of expression and activity is highly conserved in all Insects where it has been studied to date. The same pattern of expression, including the same anterior limit of the domain in the thorax, has been found for the *Ubx* gene in such different Insects as the dipteran *Drosophila*, which bears a single pair of wings, the orthopteran *Schistocerca*, which bears two wing pairs (KELSH *et al.*, 1994) , and the zygentoman (= thysanuran *sensu stricto*) *Thermobia*, which bears no wing at all (CARROLL *et al.*, 1995) . In contrast, in the crustacean branchiopod *Artemia* the pattern of expression of these *Hox* genes is quite different. *Antp*, *Ubx* and *abdA* are all expressed in the pereion (the so-called "thorax") while *AbdB* expression is restricted to the genital segments (in *Artemia*, the genitalia are located in the last two pereionic segments) (Fig. 2). Our preliminary results on Cirripedia, where the female genital aperture is located in the first pereionic segment, support the idea that *AbdB* specificity is genital rather than far-abdominal (E. MOUCHEL-VIELH & J.S.DEUTSCH, unpublished).

From these results, AVEROF and AKAM proposed a reconsideration of the homology between the different tagmata of the two arthropod classes (AKAM, 1995; AVEROF & AKAM, 1995) . In Crustacea, the segment bearing the male genitalia always marks the border between the pereion and the pleon, while the female aperture may or may not be located more anteriorly. The crustacean genital segment (or segments) would be homologous to the genital segments of Hexapoda, which are always far-abdominal (see above). The crustacean pereion would not be homologous to the hexapod thorax, but rather to the whole (thorax + abdomen) (see Fig 2).

WHAT ABOUT LEGS ?

Hexapoda are distinct from Crustacea by their terrestrial life. The terrestrial life of some crustacean species and the aquatic life of some hexapods are clearly secondary derived states. Moreover, they differ by the number of pairs of ventral appendages. All hexapods have three pairs of legs, one pair on each thoracic segment. Crustaceans never have three pereionic segments. Most have more than three, with the only exception of Ostracoda. Hence, most Crustacea have more than three pairs of legs.
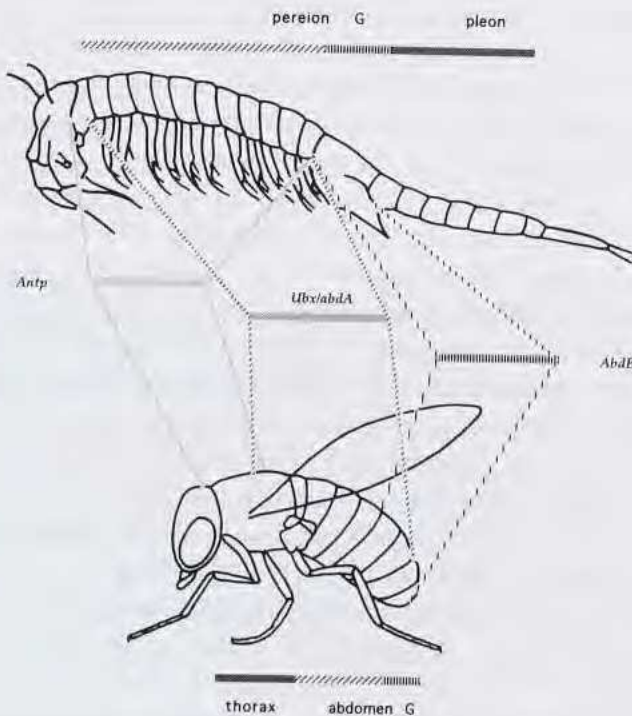
Fig. 2. — The homology between the tagmata of Crustacea and Hexapoda, according to AVEROF & AKAM (1995). The domains of expression of the *Hox* genes *Antp, Ubx, abdA* and *AbdB* are represented on a schematic drawing of *Artemia* (top) and *Drosophila* (bottom). For clarity sake, the *Hox* expression domains are represented on the body plan of an adult, although they have been observed at embryonic and/or larval stages. G : genital segments; the genital segments classically belong to the pereion in the Crustacea, and to the abdomen in the Insecta.

It is logical to assume that the body plans of these two arthropod classes are related to their eco-ethological habits: the crustacean ventral appendages are mainly used for swimming, while in a terrestrial environment it might be easier to use a small number of legs. Other terrestrian mandibulates, such as Myriapoda and the terrestrian isopod crustaceans use quite different strategies for walking: their pairs of legs are superabundant, but reduced in size, and homonomous. Although probably primarily homomous, hexapod pairs of legs are not only reduced in number, they are also different from each other, in morphology, musculature and innervation. This differentiation might well be under homeotic control. Whether these two properties (number reduction and differentiation) are correlated is an open question. Hence, this magic number of three thoracic segments and three pairs of legs in Hexapoda could be of adaptive value.

Is it possible to reconcile the absence of legs on the hexapod abdominal segments with AVEROF and AKAM's hypothesis of homology between the hexapod abdomen and a part of the crustacean pereion?

In *Drosophila* the formation of thoracic legs and cephalic appendages requires the expression of the selector gene *Distalless (Dll)*. In the thorax, the expression of *Dll* is repressed by the homeotic genes *Ubx* and *abdA* in the abdomen (VACHON *et al.*, 1992). Lack of expression

of *Ubx*, as in the *bxd* mutant, yields to the formation of an additional pair of legs, a weird fly bearing four pairs of legs (BENDER *et al.*, 1983, and references therein).

The absence of abdominal legs in Hexapoda is not such a straight rule as it seems at the first glance. Some insect nymphs and larvae possess abdominal appendages. An example is given by the so-called "prolegs" of caterpillars. In the butterfly *Precis coenia*, the formation of these prolegs has recently been studied (PANGANIBAN *et al.*, 1994) . In the embryo, the *Hox* gene *Antp* is unexpectedly expressed in a few patches of abdominal cells, where *Ubx* and *abdA* are no longer expressed. This unusual *Antp* expression is correlated with the expression of *Dll*, and the formation of the prolegs (WARREN *et al.*, 1994). Hence, the presence of abdominal legs in lepidopteran larvae is under the same genetic control that operates in the formation of thoracic legs. In that case, the change results from a subtle and localized variation in the pattern of *Hox* expression.

Another case of abdominal appendages in Hexapoda is the presence of abdominal styli in adults machilids (Archeognatha) and silver-fishes (Zygentoma = Thysanura *s. s.*). It is generally admitted that machilid styli represent a primitive trait. "The abdominal styli of machilids are generally regarded as remnants of telopodites" (BITSCH, 1994) . Fossils support the homology of these appendages with true "legs". The abdominal styli of several extinct species are composed of several articles (plurisegmented), sometimes followed by a claw. This morphology is quite similar to thoracic legs (for a review, see BITSCH, 1994).

The role of *Dll* has been recently examined in two crustaceans, the branchiopod *Artemia* and the malacostracan *Mysidopsis*. *Dll* is expressed in every branch of the developing limb, whether it is uniramous or biramous. The mode of expression of *Dll* and probably its function, as known in the uniramous insect limb, thus also applies to biramous limbs. In contrast to insects, in the pereionic limbs of *Artemia*, *Dll* is expressed in the same cells in which the *Hox* genes *Ubx/abdA* are simultaneously expressed (PANGANIBAN *et al.*, 1995). The anterior limit of expression of *Ubx/abdA* coincides in *Artemia* with the transition from maxillae to pereiopods, while its posterior limit in *Mysidopsis* corresponds to the transition from presence to absence of pleopods. In addition, the anterior limit of its expression varies in different malacostracan species studied with regard to the number of pereiopods transformed into maxillipedes (PATEL, 1995). Three conclusions can be derived from these comparative developmental genetics analyses: i) *Dll* plays the same role in the developing limb in Crustacea as in Insecta, and probably in other Arthropoda (PANGANIBAN *et al.*, 1995); ii) the *Hox* genes play a role in Crustacea as well as in Insecta in directing the morphological diversity of limbs; and iii) contrary to Insecta, *Dll* is not repressed by *Ubx/abdA* in Crustacea, and probably not regulated by any *Hox* gene.

EVOLUTIONARY SCENARIO

Summarizing these data, the following evolutionary scenario can be drawn:
i) The gross pattern of expression of *Hox* genes in *Artemia*, *i.e.* expression of *Antp*, *Ubx* and *abdA* in the pereion, *AbdB* in the genital segments, and no expression of *Hox* genes in the pleon, is primitive.
ii) Several times, various genetic mutations have occurred during the evolution of crustaceans, affecting the regulation of the *Hox* genes. These produced changes in the limits of the expression

domains of *Hox* genes. They account, at least in part, for the morphological diversity of extant and fossil Crustacea.

iii) One of these changes happened to make a distinction between the first three pereionic and following segments, thus creating a "thorax" and an "abdomen" within the pregenital region. In addition, at the same time or subsequently, subtle differentiation between the three thoracic segments was generated. These changes were fixed during the change from aquatic to terrestrial life.

iv) *Dll*, the master gene in the morphogenesis of appendages, acquired the cis-acting regulatory sequences that are a target for the products of the *Hox* genes. *Dll* thus became integrated within the panel of genes regulated by the homeotics. As a result, the abdominal legs were repressed. This does not imply any important change of the *Hox* genes themselves or of their regulation, accounting for the striking stability of the body plan within Hexapoda (including fossils).

The above hypotheses constitute a scenario, meaning that the temporal succession of the proposed events in the order i) to iv) is part of the hypotheses.

TESTS OF THE SCENARIO AND OPEN QUESTIONS

Obviously, the present scenario needs more data in order to be supported, from both phylogeny and molecular developmental genetics.

The first major open phylogenetic question is the relationship between Crustacea and Hexapoda. Morphological, developmental and molecular data have to be collected and considered in phylogenetic analyses in order to help resolve this important question.

As stressed above, the relationships between the different sub-classes and orders of the Crustacea need to be clarified, by collecting more developmental genetic information on more species. Although quite powerful, and more and more facilitated by the technological advances of molecular biology, molecular genetics is costly in time and money. It is of major importance that the species used as models in comparative developmental genetics should be selected on the basis of their phylogenetic position. This would not impair in any way the critical requirement of independence needed for phylogenetic tests of an evolutionary scenario (see GRANDCOLAS *et al.*, this volume). Once obtained, the developmental genetic data could be drawn on an independently derived phylogenetic tree.

More specifically, it is necessary to address the following questions :

To what extent does the pattern of expression of *Hox* genes as found in *Artemia* apply to other Anostraca, to other Branchiopoda, to other members of "basal" groups of Crustacea ("basal" taken here in the sense of "a lineage emerging early in evolution from the main branch")?

To what extent is this pattern different from what is observed in Malacostraca? Since the published data are incomplete, the differences reported could result from a difference in the stage at which the larvae of the two crustaceans *Artemia* and *Mysidopsis* were observed, rather than to a specific difference.

If the difference between *Artemia* and Malacostraca is confirmed, it would be of extreme importance to look for the expression of *Hox* genes in the pereion and in the pleon in Crustacea representative of other "basal" groups, in order to address the question whether this difference

correlates to greater development of muscles and nervous system in the pleon of Malacostraca as compared to other Crustacea.

What is the pattern of expression of the *Hox* genes, and in particular *abdA/Ubx*, in members of more "basal" hexapod groups, such as Archeognatha and Collembola, rather than the representatives in which it has been observed, *i.e.* in Zygentoma and Pterygota. In other words, is this pattern an apomorphy of the clade Insecta, or could it be a extended to the whole Hexapoda class?

What is the pattern of expression of *Dll* in the larval abdomen of machilids, and is it submitted to the same *Hox* control as found in other hexapods?

CONCLUSION

In the present work, I have proposed an evolutionary scenario on the origin of Hexapoda, and suggested some guidelines to test it.

Some readers may think that this type of "gross" evolutionary question is not worth dealing with. I have used this question as an example to illustrate the approach. But the same approach could be applied to a variety of evolutionary problems at any level of taxonomy. In the Hexapoda, evolutionary scenarios based on comparative developmental genetics can be drawn about such questions as the origin of the pterygote radiation (see CARROLL, 1995; CARROLL *et al.*, 1995) or the origin of the holometabolous radiation (see DEUTSCH, 1996). More specific problems can also be addressed. The number and morphology of sex combs, located on the first leg of males, is a differential character in Drosophilid species between *montium* and *melanogaster* groups. Not surprisingly, this character is genetically determined: a "montium-like" mutant of *D. melanogaster* has been isolated (F. DOCQUIER, P. SANTAMARIA, and J. S. DEUTSCH., in prep.). This example illustrates that developmental genetics might have something to tell even at the sub-genus level.

Enhanced interaction between phylogenetic analysis and evolutionary developmental genetics will not only provide more data to improve the robustness and/or consistency of the proposed phylogenetic trees, (evolutionary patterns) but in addition may give an estimation of the validity of evolutionary scenarios (processes).

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